510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k050206

B. Purpose for Submission:

Clearance of a new device

C. Measurand:

Tacrolimus

D. Type of Test:

Quantitative Colorimetric Enzymatic Immunoassay

E. Applicant:

Microgenics Corporation

F. Proprietary and Established Names:

CEDIA® Tacrolimus Assay
CEDIA® Tacrolimus Calibrators

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.1678, tacrolimus test system
   862.1150, Calibrator

2. Classification:
   Class II

3. Product code:
   MLM, enzyme immunoassay, tacrolimus
   JIT, calibrator, secondary

4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

The CEDIA® Tacrolimus Assay is an in vitro diagnostic device intended for use with automated clinical chemistry analyzers for the quantitative determination of tacrolimus in human whole blood as an aid in the management of kidney and liver transplant recipients receiving tacrolimus therapy.

CEDIA® Tacrolimus Calibrators are intended for calibration of the CEDIA® Tacrolimus Assay in whole blood.

2. Indication(s) for use:

See intended use above

3. Special conditions for use statement(s):

For professional use only

For use in kidney and liver transplant recipients receiving tacrolimus therapy

4. Special instrument requirements:

Clinical chemistry analyzers (performance characteristics determined using the Hitachi 917 analyzer)

I. Device Description:

The CEDIA® Tacrolimus Assay consists of 4 ready-to-use liquid reagents containing mouse monoclonal anti-tacrolimus analog antibodies, secondary antibodies, buffers, purified microbial enzyme acceptor, purified microbial enzyme donor conjugated to tacrolimus analog, colorimetric reagents, extraction reagents, buffers, stabilizers, and preservatives.

The CEDIA® Tacrolimus Calibrators consist of a low calibrator and a high calibrator. Human source material was tested for HIV 1 and 2, HBV and HCV with an FDA approved assay and found to be negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Abbott Laboratories IMx® Tacrolimus II (MEIA)

2. Predicate 510(k) number(s):
   P970007 (note: tacrolimus test systems have been reclassified into Class II since the predicate was approved)

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The CEDIA® Tacrolimus Assay is an in vitro diagnostic medical device intended for the quantitative determination of tacrolimus in human whole blood using automated clinical chemistry analyzers as an aid in the management of kidney and liver transplant recipients receiving tacrolimus therapy. CEDIA® Tacrolimus Calibrators are intended for calibration of the CEDIA® Tacrolimus Assay in whole blood.	The IMx® Tacrolimus II assay is an in vitro reagent system for the quantitative determination of tacrolimus and some metabolites in human whole blood and as an aid in the management of liver allograft patients receiving tacrolimus therapy. The IMx® Tacrolimus II Calibrators are for calibration of the IMx® analyzer when used for the quantitative determination of tacrolimus in human whole blood.
Analyte	Tacrolimus	Tacrolimus
Matrix	Whole blood	Whole blood

Differences		
Item	Device	Predicate
Patient Population	Kidney and liver transplant patients	Liver transplant patients
Calibrator	Two levels	Six levels

K. Standard/Guidance Document Referenced (if applicable):

CLSI Document EP5-A - Evaluation of Precision Performance of Clinical Chemistry Devices

L. Test Principle:

The CEDIA® Tacrolimus Assay is based on β-galactosidase which has been expressed in two inactive fragments (donor and acceptor) that can re-associate in solution to create active enzyme. Drug in the specimen competes with tacrolimus analog that is conjugated to the enzyme donor for anti-tacrolimus antibody binding sites (which are limited). When antibody binds drug in the sample, the enzyme donor molecules are free to bind the enzyme acceptor molecules to form active β-galactosidase. The active enzyme cleaves a colorimetric substrate. The amount of active enzyme formed (and the resultant absorbance change) is directly proportional to the amount of drug in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

Assay imprecision was evaluated according to CLSI EP5-A. Three (3) whole blood pools from patients taking tacrolimus and 3 negative whole blood pools spiked with tacrolimus to targeted concentrations of 5, 10, and 20 ng/mL were assayed in 21 runs over 11 days. Each pool was measured multiple times to observe 126 data points for each pool. Results are summarized below (units = ng/mL).

		Within Run		**Total**	
	Mean	**SD**	**% CV**	**SD**	**% CV**
Low	6.30	0.43	6.80 %	0.49	7.80 %
Medium	9.23	0.43	4.68 %	0.56	6.06 %
High	15.18	0.47	3.12 %	0.65	4.26 %
Spiked Low	5.33	0.32	5.97 %	0.48	8.90 %
Spiked Medium	10.54	0.35	3.28 %	0.49	4.69 %
Spiked High	21.01	0.43	2.04 %	0.72	3.40 %

Spiked recovery was evaluated by spiking 4 negative whole blood samples with 5, 10, 15, or 25 ng/mL tacrolimus. The spiked samples were each assayed 21 times and the observed concentration was compared to the expected concentration. Results are summarized below (units = ng/mL).

Expected	**Observed**			
	Mean	**SD**	**% CV**	**% Recovery**
5	5.05	0.54	10.7 %	101.0 %
10	9.65	0.48	5.0 %	96.5 %
15	15.78	0.60	3.8 %	105.2 %
25	25.31	0.46	1.8 %	101.2 %

b. Linearity/assay reportable range:

To evaluate the linearity of the assay, 3 patient whole blood samples with a high concentration of tacrolimus were serially diluted using a negative whole blood sample. The samples were assayed in quadruplicate over three runs. The results are summarized below (units = ng/mL).

Sample 1

Dilution	Expected	Observed	% Recovery
100 %	34.3	34.3	100.0 %
80 %	27.4	27.6	100.8 %
60 %	20.6	21.4	104.0 %
40 %	13.7	15.1	110.0 %
20 %	6.9	6.7	97.8 %
0 %	0.0	0.0	---
Regression	Observed = 1.003(Expected) + 0.343; r = 0.9988		

Sample 2

Dilution	Expected	Observed	% Recovery
100 %	28.5	28.5	100.0 %
80 %	22.8	23.2	102.0 %
60 %	17.1	18.2	106.7 %
40 %	11.4	12.2	107.3 %
20 %	5.7	5.6	97.9 %
0 %	0.0	0.0	---
Regression	Observed = 1.033(Expected) - 0.231; r = 0.9983		

Sample 3

Dilution	Expected	Observed	% Recovery
100 %	28.9	28.9	100.0 %
80 %	23.1	23.9	103.4 %
60 %	17.3	18.1	104.7 %
40 %	11.5	12.4	107.7 %
20 %	5.8	6.1	106.2 %
0 %	0.0	0.0	---
Regression	Observed = 1.008(Expected) + 0.347; r = 0.9992		

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The CEDIA® Tacrolimus Calibrators are ready-to-use liquid calibrators prepared in a whole blood matrix. The calibrators contain ascomycin rather than tacrolimus. Ascomycin is an ethyl analog of tacrolimus and differs at only one position (position 21). The primary antibody in the CEDIA® Tacrolimus Assay is 100% reactive with both tacrolimus and ascomycin.

Primary calibration standards are gravimetrically prepared using tacrolimus to targeted values of 0 and 30 ng/mL. Primary standards are verified by testing multiple replicates of each level in multiple analyzer runs. Concentrations are also independently validated using LC-MS/MS. The kit calibrators are prepared gravimetrically from purified ascomycin. The primary standards are used to value-assign the kit calibrators multiple times in multiple runs. The target values for the low and high calibrators are 0 and 30 ng/mL, respectively.
Real-time and accelerated stability of the kit components was used to determine a claim of 24 months (refrigerated reagents and frozen calibrators).

d. Detection limit:

The functional sensitivity of the assay (defined as the concentration at which interpolated % CV is 20%) was evaluated. Patient samples containing tacrolimus were pooled and aliquots were diluted using negative whole blood to obtain pools containing 1, 2, 3, 4, and 5 ng/mL tacrolimus. The pools were measured in triplicate in 10 runs, and the % CV was plotted versus tacrolimus concentration. The functional sensitivity of the assay is 2.0 ng/mL.

To determine the analytical sensitivity of the assay, 21 replicates of the low calibrator (0 ng/mL analyte) and 21 replicates of negative whole blood samples were assayed over 3 separate runs. The analytical sensitivity (calculated as 2 standard deviations above the observed average) for the calibrator and the negative whole blood was 0.8 ng/mL and 1.2 ng/mL, respectively. The claimed analytical sensitivity, reported as the highest concentration reading observed during the 3 runs, is 1.5 ng/mL tacrolimus.

e. Analytical specificity:

Potential interferences were evaluated by adding known amounts of exogenous and endogenous substances to negative whole blood samples. The samples were controlled with matched samples spiked with solvent only. Five replicates of each sample were assayed and the % recovery was calculated. The results are summarized below.

Interferant		**Tacrolimus (ng/mL)**		
		Control Sample	**Test Sample**	**Recovery**
Uric Acid	20 mg/dL	9.42	8.85	94.0 %
Triglycerides	1500 mg/dL	17.20	16.90	98.3 %
Cholesterol	500 mg/dL	15.99	15.69	98.1 %
Bilirubin	60 mg/dL	17.53	17.25	98.4 %
γ-globulin	12 g/dL	16.29	15.85	97.3 %
Albumin	12 g/dL	16.49	16.58	100.6 %
Hematocrit	18.4 %	11.3	11.46	101.6 %
	24.5 %		11.95	106.0 %
	35.1 %		11.28	> 99 %
	40.4 %		10.75	95.3 %
	51.2 %		12.21	108.3 %

	59.6 %		11.32	100.3 %
Rheumatoid Factor	573 IU/mL	18.60	20.12	108.1 %
EDTA	4.5 ng/mL	15.06	14.07	93.4 %

Potential cross-reactivity with tacrolimus metabolites was evaluated by adding 20 ng/mL of the major metabolites of tacrolimus to negative whole blood samples. Five replicates of each sample were assayed and the cross-reactivity was calculated. The results below indicate that the assay cross-reacts to 13-O-desmethyl tacrolimus. Therefore, results from this assay may be slightly higher than those obtained with HPLC-MS/MS. There is a potential bias between the two methods and the labeling will emphasize the need to establish a clinical range for the CEDIA® Tacrolimus Assay. (units = ng/mL)

	Observed	**Cross-reactivity**
13-O-desmethyl tacrolimus	7.53	37.7 %
31-O-desmethyl tacrolimus	0.98	4.9 %
15-O-desmethyl tacrolimus	0.95	4.7 %
13-31 –O-didesmethyl tacrolimus	0.57	2.9 %

Potential cross-reactivity by other common immunosuppressants was evaluated by adding known amounts of the various immunosuppressants to negative whole blood samples with and without tacrolimus. The samples were controlled with matched samples spiked with solvent only. Three replicates of each sample were assayed and the cross-reactivity was calculated. The results are summarized below. (units = ng/mL)

Drug		**Expected Assay Result**	**Observed Assay Result**	**Cross-reactivity**
Cyclosporine	10,000	0.0	0.4	0 %
		18.7	18.8	0 %
Mycophenolic acid	100,000	0.0	0.0	0 %
		20.5	19.8	0 %
Sirolimus	300	0.0	0.6	0.20 %
		15.3	18.4	1.03 %
Sirolimus	100	0.0	0.7	0.7 %
		16.1	16.5	0.4 %
Sirolimus	50	0.0	0.6	1.2 %
		17.4	17.6	0.4 %
Sirolimus	30	0.0	0.2	0.67 %
		16.7	17.1	1.3 %

Potential cross-reactivity from common pharmacologic substances was evaluated by adding potentially interfering drugs to tacrolimus-negative whole blood samples. No cross-reactivity was observed for any of the compounds. The complete list of compounds tested can be found in the assay package insert.

f. Assay cut-off:

Not applicable

2. Comparison studies:
   a. Method comparison with predicate device:

Tacrolimus concentrations were determined for trough patient samples using both the CEDIA® Tacrolimus Assay and conventional high performance liquid chromatography with mass spectrometry detection (LC-MS/MS). Samples provided for method comparison studies were obtained post-operatively from renal and hepatic allograft recipients. All samples were trough draws.

An internal evaluation at the sponsor's site used samples provided by a US multi-organ transplant center and a European medical research university hospital. Data on a portion of those samples using the predicate deice (assay 1) was generated at the US multi-organ transplant center.

Eternal evaluations were performed at two transplant centers in Australia and New Zealand and in an Australian clinical laboratory. Samples originated at the two transplant centers.

The linear regression statistics are summarized below. These results show that there is a predictable, expected bias between the two methods; with the CEDIA® Tacrolimus Assay results being somewhat higher than those obtained using LC-MS/MS. This information will be included in the labeling with a caution that users should establish their own reference ranges and not compare values between methods.

Study	Transplant Type	n	Slope	Intercept	Correlation (r)
Microgenics Evaluation					
CEDIA vs. LC-MS/MS	Kidney and Liver	187	1.190 (1.144 to 1.235)	0.70 (0.35 to 1.05)	0.9643
CEDIA vs. LC-MS/MS	Kidney	118	1.157 (1.112 to 1.202)	1.01 (0.66 to 1.35)	0.9777
CEDIA vs. LC-MS/MS	Liver	69	1.193 (1.113 to 1.274)	1.04 (0.40 to 1.67)	0.9616
CEDIA vs. Assay 1	Liver	50	0.945 (0.781 to 1.109)	0.28 (-1.42 to 1.99)	0.8228
External Evaluation					
CEDIA vs. LC-MS/MS	Kidney and Liver	176	1.267 (1.193 to 1.341)	-0.78 (-1.45 to –0.11)	0.9230
CEDIA vs. Assay 1	Liver	75	1.094 (1.004 to 1.185)	0.22 (-0.67 to 1.12)	0.9370

   b. Matrix comparison:

Not applicable

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable

   b. *Clinical specificity**:***
      Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):
      Not applicable

4. Clinical cut-off**:**
   Not applicable

5. Expected values/Reference range:

The recommended range of tacrolimus concentrations in whole blood for effective post-operative management of kidney and liver allograft transplant patients is 5 ng/mL to 20 ng/mL **using LC/MS**. The optimal therapeutic range for tacrolimus in whole blood has not been established with this assay.

Optimal tacrolimus concentration ranges vary according to the methodology used, and therefore should be established for each commercial test. Values obtained with different assay methods cannot be used interchangeably due to differences in cross-reactivity with metabolites, nor should correction factors be applied. Laboratories should include identification of the assay used in order to aid in interpretation of results. Tacrolimus concentrations for individual patients should be determined using a single, consistent method to minimize confounding effects associated with cross-reactivity and recognition of metabolites.

The patient's current and past clinical state, individual differences in sensitivity to immunosuppressive and toxic effects of tacrolimus, co-administration of other immunosuppressants, time post-transplant, and a number of other factors may cause different requirements for optimal blood concentrations of tacrolimus. Therefore, individual tacrolimus values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each institution should establish the optimal ranges based on the specific assay used and other factors relevant to their patient population.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.